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TECHNICAL MANUSCRIPT 452

GROWTH OF RIFT VALLEY FEVER VIRUS IN HUMAN DIPLOID (WI-38) CELLS

Roger W. Johnson
Michael D. Orlando
William C. Patrick, III

MARCH 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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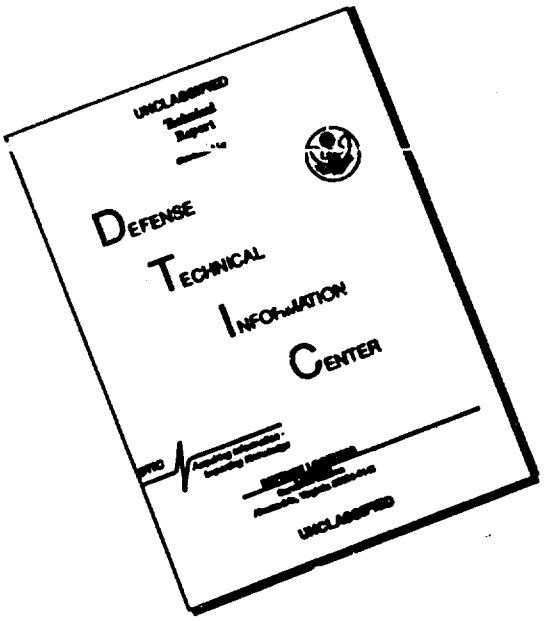

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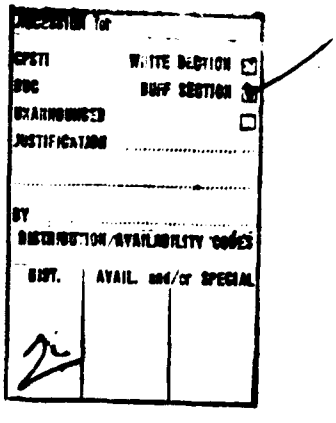

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Product Development Division
AGENT DEVELOPMENT AND ENGINEERING LABORATORY

Project 1B533001D426

March 1968

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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We acknowledge the technical assistance of Mr. Jerry Keller.

ABSTRACT

The growth of Rift Valley fever virus in human diploid (WI-38) cells was studied. Peak titers of $1 \times 10^{9.0}$ and $1 \times 10^{9.4}$ mouse intracerebral median lethal doses per milliliter were obtained in monolayer culture and suspensions, respectively.

GROWTH OF RIFT VALLEY FEVER VIRUS IN HUMAN DIPLOID (WI-38) CELLS

The growth of Rift Valley fever (RVF) virus in established cell lines[1,2] and in primary cells[3,4] has been described. The present report describes the growth of RVF virus in the human diploid strain WI-38.[5]

We used strain WI-38 cells in the 22nd passage,* either as monolayer cultures in 32-oz glass prescription bottles or in suspension in Eagle's basal medium[6] supplemented with 10% calf serum.

The Van Wyk pantropic strain of RVF virus,[7] passed once in tissue culture from infected lamb serum and stored at -80 C, was used.

Washed monolayer cultures were infected with enough RVF virus in 10-ml amounts to give an input multiplicity of 15 $MICLD_{50}$ per cell. After 1 hour adsorption at 37 C, the inoculum was poured off. The cultures were then washed three times, and 80 ml were added of either Eagle's basal medium or medium 199,[8] both supplemented with 10% calf serum and 50 μg kanamycin**/ml. The cultures were incubated at 37 C and assayed periodically for virus by mouse intracerebral inoculation.

Suspensions of WI-38 cells, adjusted to a density of about 6×10^5 cells/ml in 500-ml volumes, were placed in 1-liter cylinders fitted with New Brunswick fermentor heads (impeller housing, vent and sample line). These suspensions were infected after 10 minutes' agitation (impeller speed 100 to 125 rpm) with enough virus to yield an input multiplicity of about 60 $MICLD_{50}$ per cell. The cultures were sampled periodically for cell count and virus assay.

In monolayer culture (Fig. 1), after an initial period of about 6 hours characterized by little increase in viral titer, the titer increased rapidly, reaching a peak of about $1 \times 10^{9.0}$ $MICLD_{50}$/ml by 30 hours. The titer then remained constant through the 72nd hour, the last time the cultures were assayed. Neither Eagle's basal medium nor medium 199 had any appreciable effect on virus growth in the WI-38 cells.

In suspensions of WI-38 cells (Fig. 2), after a lag of about 6 hours, viral titer increased to a peak of about $1 \times 10^{9.4}$ by 27 hours. The titer remained constant until the 76th hour, after which it decreased. The viable cell counts decreased over the course of the study, reaching zero by 94 hours postinfection.

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* Received from Flow Laboratories, Inc., Rockville, Md.
** Kantrex, Bristol Laboratory, Syracuse, N.Y.

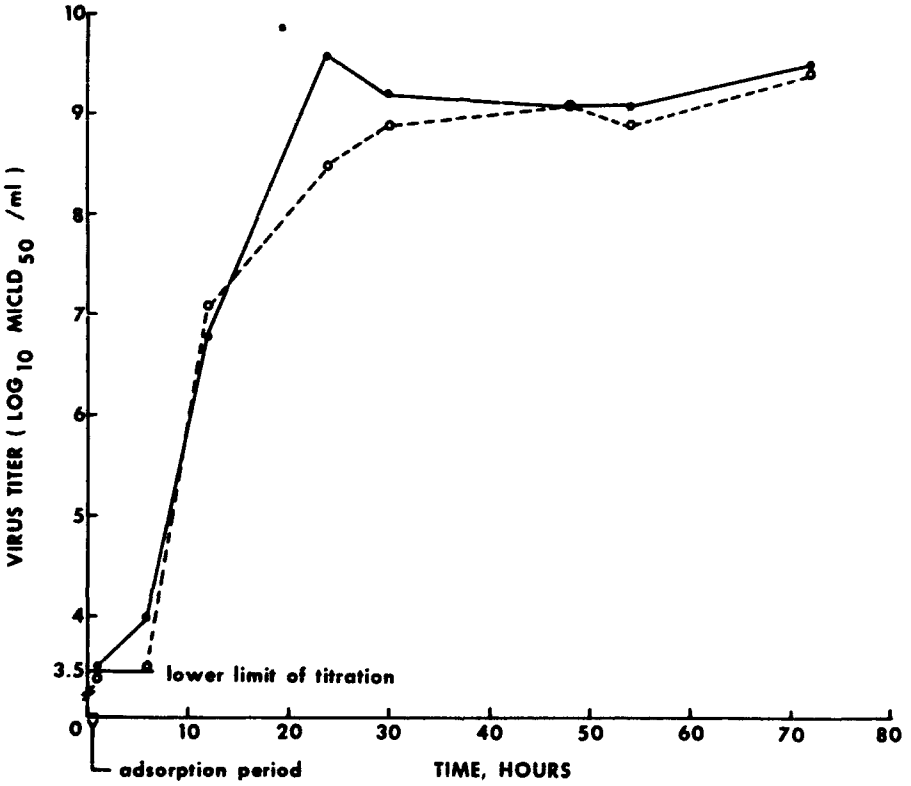

Figure 1. Growth of Rift Valley Fever Virus in Human Diploid Cell Monolayer Culture. ●—● = Growth in Eagle's basal medium; O--O = growth in medium 199; both media were supplemented with 10% calf serum and 50 μg kanamycin/ml.

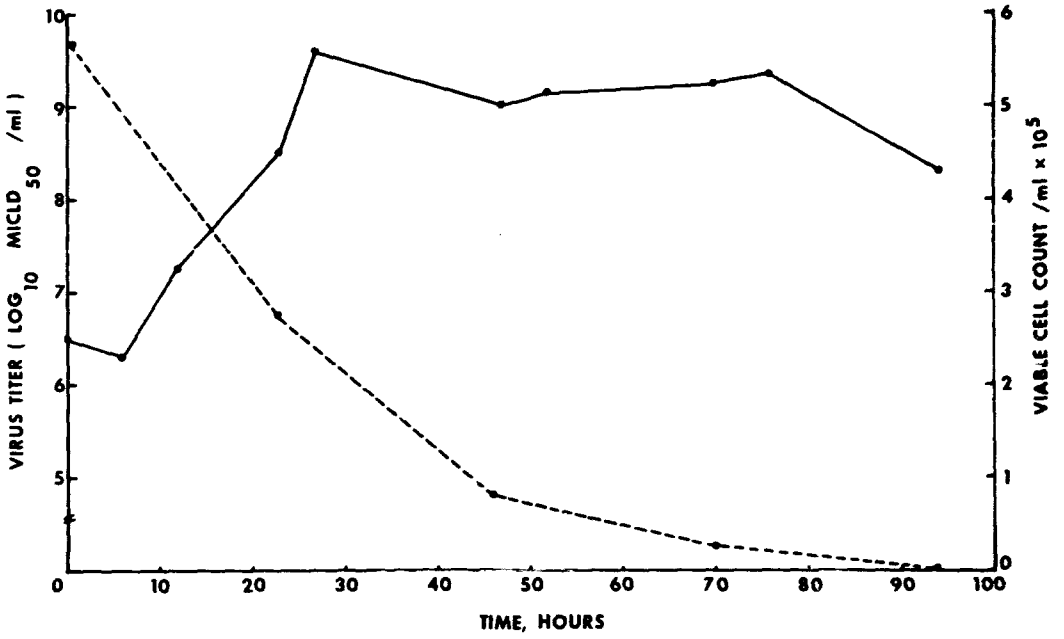

Figure 2. Growth of Rift Valley Fever Virus in Suspended Human Diploid Cells. ●—● = Virus titer; O--O = viable cell count.

The growth curves of RVF virus in monolayer cultures and suspensions of WI-38 cells were essentially the same as those for RVF virus in monolayer and suspension cultures of mouse fibroblast-like (MFL) cells.[3] The titer of the virus in the suspended WI-38 cells remained high longer than it did in suspensions of MFL cells[3] (50 hours versus 15 hours from peak, respectively). This could be caused in part by the difference in the media used in the two studies (Eagle's basal medium in the present study, medium 199 in the previous) and by a difference in impeller speeds (100 to 125 rpm in the present study compared with about 250 to 300 rpm in the previous study).

The viability of the infected WI-38 cells in suspension followed a course similar to that of the infected MFL cells in suspension.

Should serially transferred cell strains ever be approved for use in vaccine production, the methods of growing virus described in this report should prove easier and more economical and provide a more standard cell population than do the primary cells currently used for RVF virus vaccine production.[4]

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13. ABSTRACT

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14. Key Words

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